

## The Importance of Aerobic Fitness in Extending Thermotolerance in Extreme Environments: Connecting Molecular Biology to the Whole Body Response

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### ABSTRACT

**Introduction/Relevance** Current theatres of military operations involve exposure to high ambient temperatures while wearing protective clothing such as fragmentation vests and/or the use of biological and chemical ensembles. Under conditions of uncompensable heat stress, aerobic fitness is a critical factor in explaining the higher core temperature ( $T_c$ ) that can be tolerated by endurance trained (TR) versus untrained (UT) individuals. Tolerating higher  $T_c$  not only extends exposure time but it ensures that individuals can safely continue to be mobile while carrying their own weight and additional loads. **Rationale** The purpose of the current research was to examine whether differences in the immuno-inflammatory activation existed between TR and UT that might help explain the different  $T_c$  tolerated at exhaustion (EXH). **Methods** Twelve TR ( $24 \pm 1$  y,  $73.3 \pm 2.2$  kg,  $9.6 \pm 1.0$  %fat,  $62 \pm 2$  ml/kg/min  $VO_{2peak}$ ) and eleven UT ( $23 \pm 1$  y,  $78.7 \pm 2.7$  kg,  $15.8 \pm 1.7$  %fat,  $42 \pm 1$  ml/kg/min) walked at 4.5 km/h and a 2% grade to EXH in  $40^\circ\text{C}$  and 30% RH while wearing a biological and chemical protective overgarment. Blood was sampled at  $0.5^\circ\text{C}$  increments until  $T_c$  reached  $40.0^\circ\text{C}$  or the trial was terminated due to high heart rates, nausea, dizziness or volition. Plasma endotoxin and serum lipopolysaccharide binding protein (LBP) were determined as were intracellular pro- and anti-inflammatory cytokines and heat stress protein (HSP) in circulating monocytes using flow cytometry. Blood volume (BV) was determined before the heat stress trial using indocyanine-green<sup>TM</sup>. **Results** There were no differences between groups in the rate of  $T_c$  increase ( $1.2 \pm 0.1^\circ\text{C/h}$ ). Thus, the significantly longer tolerance time for TR ( $163 \pm 11$  min) vs UT ( $106 \pm 10$  min) reflected the differences in  $T_c$  tolerated at EXH ( $39.7 \pm 0.1^\circ\text{C}$  vs  $39.1 \pm 0.1^\circ\text{C}$  for TR and UT, respectively). BV was significantly increased for TR ( $104 \pm 6$  mL/kg) vs UT ( $84 \pm 3$  mL/kg) but these differences were not related ( $r = 0.35$ ) to the differences in  $T_c$  tolerated. Exercise and heat stress invoked significant increases in plasma endotoxin and LBP but the increase was greater for UT vs TR. Plasma endotoxin was more than 2-fold greater for UT than TR at  $39.0^\circ\text{C}$  and at EXH. Pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  in inflammatory monocyte subsets increased for both groups with increasing  $T_c$  yet values were greater for TR compared with UT. However, at higher  $T_c$  these cytokine expressions decreased for TR but continued to increase for UT. Anti-inflammatory cytokines IL-6 and IL-1ra were also increased for TR vs UT. Intracellular HSP expression increased with increasing  $T_c$  for TR only. At EXH, the percentage of spontaneous apoptotic cells were significantly increased for UT whereas no change was observed for TR compared to baseline. **Conclusion** These findings suggest a reduced intracellular inflammatory activation in TR, and an impaired HSP induction and increased heat-induced cellular apoptosis in UT providing a connection between molecular protein expression and enhanced thermotolerance with endurance training.

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## **1.0 INTRODUCTION**

Current military deployments involve exposure to hot environments together with the requirement to wear protective body armour and/or additional clothing layers to protect against the threat of exposure to biological and chemical (BC) agents. Although the garments confer protection for the soldier from the hazards of their operating environment, the clothing together with the hot temperatures creates a condition of uncompensable heat stress [for review see 5] where thermoregulation is not possible and where the continued rise in core temperature places the soldier at an increasing risk of becoming a heat casualty.

The pathophysiology of exertional heat illness (EHI) is not fully understood, but due to a number of similarities between the symptoms of heat stroke and those of endotoxic shock, a common belief has developed that gut-derived endotoxins are a primary mediator of heat stroke [2, 9]. Heat stress exposure involves redistribution of blood flow away from the splanchnic tissues to assist with the transfer of internal heat to the skin surface [16]. As core temperature continues to rise, so does the redistribution of blood away from the splanchnic area leading to an eventual break down in the intestinal barrier integrity and leakage of endotoxin from the gut into the portal and systemic circulations. There is a strong contention that endotoxemia is a key mediator driving the systemic inflammatory response-related progression of EHI at temperatures greater than 41°C [2]. However, whether these mechanisms are also occurring during exertional heat stress (EHS) and are responsible for the progressions of EHI severity at core temperatures below 40°C are less conclusive.

It is now well documented that endurance trained (TR) individuals can safely tolerate higher core or rectal temperatures ( $T_{re}$ ) than their untrained (UT) counterparts [4, 14, 18, 19], thus extending their heat storage capacity and their range of thermotolerance before succumbing to exertional heat injury. The increased blood volume that accompanies aerobic training [8] enhances cardiovascular stability at any given level of thermal strain, which by itself might explain the ability to tolerate higher  $T_{re}$ . However, animal models suggest that endotoxemia also plays an important role [17]. Thus it is possible that the improved cardiovascular stability associated with aerobic training impacts endotoxin translocation, inflammatory activation and heat tolerance. Therefore, the purpose of the present study was to examine whether there is evidence for immuno-inflammatory activation due to endotoxin leakage that is consistent with a continued increasing level of thermal strain during EHS and that is consistent with the proposed cytoprotective effects conferred by an increased level of aerobic fitness.

## **2.0 METHODS**

### **2.1 Subjects**

Following approval by the Defence Research and Development Canada (DRDC) and York University Human Research Ethics Committees, 23 healthy males were recruited from surrounding universities and running clubs in the greater Toronto area. All subjects were medically screened and a full explanation of procedures, discomforts, and risks were given prior to obtaining written informed consent. Potential subjects were excluded if they did not fit the grouping criteria described below or if they were taking any medications. In addition, subjects were screened for a history of allergy to iodides, and/or sensitivities to penicillin and sulfa drugs, a contraindication for ICG dye injection. All testing was performed in an exercise laboratory or a climatic chamber at DRDC Toronto.

## 2.2 Determination of $\dot{V}O_{2peak}$

$\dot{V}O_{2peak}$  was determined on a motor-driven treadmill using open-circuit spirometry [12] before the series of experiments in the climatic chamber. Following three minutes of running at a self-selected pace, the treadmill grade was increased 1%/min until subjects were running at a 10% grade. If necessary increases in treadmill speed of 0.22 m/s and grade of 1% were alternated each minute until the subject could no longer continue.

$\dot{V}O_{2peak}$  was defined as the highest oxygen consumption ( $\dot{V}O_2$ ) observed during the incremental test. Heart rate (HR) was monitored throughout the incremental test from a telemetry unit (Polar Electro PE3000, Stamford, CT). The HR value recorded at the end of the exercise test was defined as the individual's peak value ( $HR_{peak}$ ). Body fatness was calculated using body density determined using underwater weighing, and a residual lung volume determined using body plethysmography. Lean body mass (LBM) was calculated by subtracting body fat from the total body mass.

## 2.3 Grouping Criteria

Subjects were divided into two groups of endurance TR (n=12) or UT (n=11) individuals based on  $\dot{V}O_{2peak}$  expressed relative to LBM and activity profiles. TR were defined as actively participating in a cardiovascular training program more than 3 times per week and having a  $\dot{V}O_{2peak}$  greater than  $65 \text{ mL} \cdot \text{kgLBM}^{-1} \cdot \text{min}^{-1}$ . UT were defined as being minimally active (less than 2 times per week) and having a  $\dot{V}O_{2peak}$  below  $50 \text{ mL} \cdot \text{kgLBM}^{-1} \cdot \text{min}^{-1}$ .

## 2.4 Experimental Design

All subjects participated in both a familiarization and experimental EHS session, which began at ~8:00 am. Familiarization sessions involved exposure to all dressing procedures (including venous catheter and rectal temperature probe insertion) as well as a brief heat exposure while walking in the climatic chamber. Each familiarization session was performed at least 10 days prior to the experimental trial to limit the acute effects of heat acclimation. In addition, the familiarization heat exposure was limited to 30 minutes in order to maintain a  $T_{re}$  below  $38.0^\circ\text{C}$ . These restrictions on increases in  $T_{re}$  were primarily for UT subjects, since TR subjects continued their regular training regiments leading up to the experimental EHS. Subjects refrained from strenuous exercise (running, swimming, cycling, weight lifting, etc.), alcohol, and the use of nonsteroidal anti-inflammatory drugs for 24 hours before and the use of caffeine for 8 hours before each session.

## 2.5 Exertional Heat Stress Model

During the familiarization session and EHS trial, subjects walked on a motorized treadmill ( $4.5 \text{ km} \cdot \text{h}^{-1}$ , 2% incline, wind speed  $<0.1 \text{ m} \cdot \text{s}^{-1}$ ) in an environmental chamber ( $40^\circ\text{C}$ , 30% R.H.) while wearing the Canadian Forces NBC protective garment over combat clothing, shorts, and a T-shirt. The intensity of exercise was selected such that the elevated metabolic rate, together with the clothing ensemble, created an uncompensable EHS condition and produced exposure times of approximately two and three hours for UT and TR, respectively. Exhaustion (EXH), which was defined by specific end-point criteria for the EHS, included an ethical  $T_{re}$  cut-off of  $40.0^\circ\text{C}$ , HR reaching or exceeding 95% of maximum for three consecutive minutes, EXH/thermal discomfort, dizziness or nausea precluding further participation, and/or subject or experimenter termination. Tolerance time (TT) was defined as the elapsed time from the beginning of walking on the treadmill to the attainment of one or more of the end-point criteria that resulted in termination of the EHS trial.

## **2.6 Physiological Measurements**

Heart rate was monitored using a transmitter attached with an elasticized belt fitted around the chest and taped in place. The receiver was taped to the outside of the clothing, allowing for a continuous HR display. Open-circuit spirometry was used to determine expired minute ventilation,  $\dot{V}O_2$ , and carbon dioxide production at each  $T_{re}$  increment from values averaged over a 2-min sampling period.  $T_{re}$  was measured using a flexible vinyl-covered rectal thermistor inserted approximately 15 cm beyond the anal sphincter. Mean skin temperature ( $M_{sk}$ ) was obtained from seven temperature thermistors taped to the head, abdomen, medial deltoid, hand, anterior thigh, shin, and foot, respectively. Mean values over 1-min periods for  $T_{re}$ , and a 7-point weighted  $M_{sk}$  were calculated as described previously [12], recorded, and printed by the computerized data-acquisition system. Subjects received 5 mL·kgLBM<sup>-1</sup> of warm water (37°C) prior to entering the climatic chamber and following metabolic measurements approximately every 30 minutes during the trial in order to limit heat-sink effects, reduce circulatory instability produced by progressive dehydration, and to increase the level of  $T_{re}$  tolerated at EXH. Sweat rate (SR) was calculated using values for nude masses pre and post-exposure, fluid administered, and TT; SR values were corrected for respiratory and metabolic mass losses as described previously [12]. Hematocrit and hemoglobin values were determined using a hematology analyzer and used to correct circulating concentrations for changes in blood and plasma volumes using equations from Dill and Costill [6].

## **2.7 Blood Collection and Storage**

Venous whole blood was collected at 6 sampling times during the experimental EHS session, if available, using an indwelling venous catheter and a 24-inch extension which protruded from the sleeve of the protective ensemble. Following catheter insertion, subjects remained standing for 20 min to obtain postural stability prior to the baseline sample. Subsequent samples were taken during the experimental heat stress trial at specific  $T_{re}$  intervals (38.0°C, 38.5°C, 39.0°C, 39.5°C and 40°C/EXH) rather than at specific time periods. Catheter patency was maintained by injecting 3 mL of a sterile saline solution between sampling intervals. Blood samples were drawn into sterile syringes and immediately transferred into corresponding vacutainers containing sodium heparin for flow cytometry analyses and K<sub>2</sub>EDTA for hematology analyses. K<sub>2</sub>EDTA plasma samples for endotoxin determination were always collected first under strict aseptic techniques with certified endotoxin free tips and storage containers. The remaining supernatants were then transferred into polypropylene Eppendorf tubes. Samples were frozen and stored at -70°C until assayed.

## **2.8 Determination Blood and Plasma Volumes**

Blood (BV) and plasma volumes (PV) were determined prior to the familiarization and experimental EHS trials using standard laboratory dilution techniques with indocyanine-green<sup>TM</sup> (ICG) [13]. Blood flow was occluded for 2 min prior to infusion of 0.25 mg·kg<sup>-1</sup> ICG<sup>TM</sup> into one arm followed by repeated sampling from the other arm from minutes 2 to 11. Individual ICG<sup>TM</sup> dye dilution curves were constructed and extrapolated back to time 0 to calculate PV from measured Hct.

## **2.9 Chromogenic *Limulus* Amebocyte Lysate and Lipopolysaccharide Binding Protein Assays**

Circulating plasma levels of endotoxin were measured using a Limulus Amebocyte Lysate Chromogenic Assay (LAL; Associates of Cape Cod, Inc., East Falmouth, MA). Maximum sensitivity of the chromogenic assay was 0.005 Eu·mL<sup>-1</sup>. All labware and storage containers were periodically tested for endotoxin

absorption and assay enhancement/inhibition.

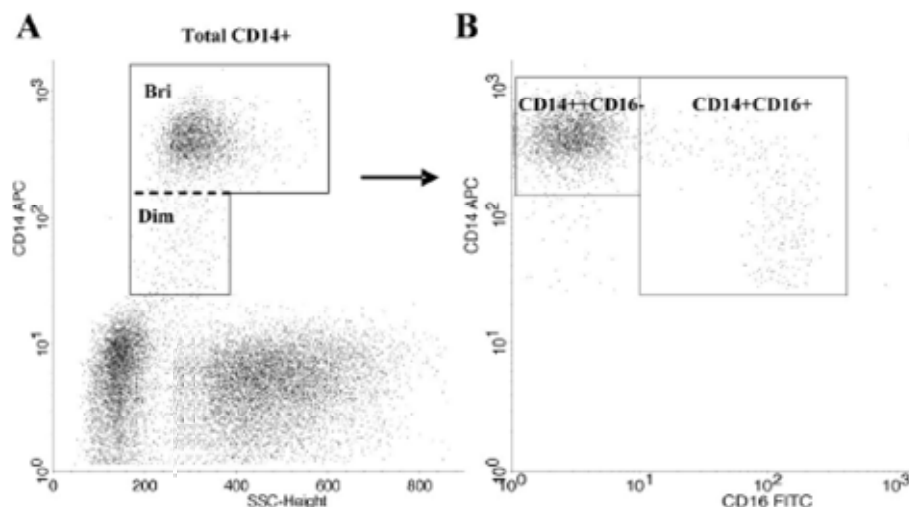
Lipopolysaccharide binding protein (LBP) was measured using a chemiluminescent immunometric assay performed on an IMMULITE® system (Diagnostic Products Corp., Los Angeles, CA, USA). The detectable limit of the assay was  $0.2 \mu\text{g}\cdot\text{mL}^{-1}$ .

## 2.10 Intracellular Cytokines, HSP72 and Apoptosis

Brefeldin A-treated and nontreated heparinised whole blood samples were analyzed immediately using flow cytometric techniques for spontaneous intracellular cytokine (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-1ra, and IL-10) and HSP72 production as well as surface staining for apoptotic markers as previously described [20]. In addition, at baseline and EXH blood was *in vitro* heat shocked in a water bath at 42°C for 2 h for HSP72 production and cellular apoptosis.

## 2.11 Flow Cytometry

All samples were acquired on a FACSCalibur flow cytometer calibrated for four-colour analyses and analyzed using Cell Quest Pro software (Becton Dickinson Biosciences, San Jose, CA). Quadrant markers and analysis gates for delineation of positive and negative regions were based on isotype-matched or negative controls. Additional dot plots gating on CD14<sup>+</sup> monocytes were used to delineate classic (CD14<sup>++</sup>CD16<sup>-</sup>) and inflammatory monocyte (CD14<sup>+</sup>CD16<sup>+</sup>) subsets (Figure 1).



**Figure 1: Flow cytometric dotplots representing sequential gating for CD14++CD16-/ CD14+CD16+ and CD14Bri/CD14Dim subsets of total CD14+ monocytes in lysed whole blood. Subsequent analyses utilized dual-color dotplots for cellular apoptosis and fluorescence histograms for intracellular HSP72 in CD14Bri/CD14Dim and intracellular cytokine staining in CD14++CD16-/ CD14+CD16+ (data not shown).**

## 2.12 Data Analyses

An ANOVA with one repeated factor (temperature) and one between factor (fitness) was calculated on the various immunologic and physiologic dependent measures sampled during EHS as well as a separate ANOVA comparison between baseline and EXH measures. In addition, a one-factor (fitness) ANOVA was used to compare physiologically dependent measures, such as  $T_{re}$  tolerated at EXH, TT, rate of  $T_{re}$  increase, and anthropometric data. An ANOVA with two repeated factors (temperature and stimulation or shock or subset) and one between factor (fitness) was calculated on baseline and EXH measures, *in vitro* samples stimulated with LPS, or heat shocked (HS), respectively. For all analyses, unless otherwise specified, subject numbers were as follows: Baseline to 38.5°C and EXH n=TR (12), UT (11); 39.0°C n=TR (12), UT (9); 39.5°C n=TR (11). To correct for violations in the assumption of sphericity with the repeated factors, we applied the Huynh-Feldt correction to the F-ratio. Post-hoc comparisons were performed using a Newman-Keuls procedure to isolate specific between-group mean differences at each  $T_{re}$  interval and within-group differences over temperature. For all analyses, an alpha level of 0.05 was used.

## 3.0 RESULTS

### 3.1 Group Characteristics

$\dot{V}O_{2peak}$  expressed per kg of LBM ( $70 \pm 2$  vs  $50 \pm 1$  ml·kgLBM<sup>-1</sup>·min<sup>-1</sup>) and per kg of total body mass ( $62 \pm 2$  vs  $42 \pm 1$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) as well as body fatness ( $9.6 \pm 1.0$  vs  $15.8 \pm 1.7$  %), BV ( $104.2 \pm 5.7$  vs  $84.0 \pm 3.1$  ml·kg<sup>-1</sup>), and PV ( $64.4 \pm 3.6$  vs  $51.0 \pm 1.9$  ml·kg<sup>-1</sup>) were significantly different between groups (TR vs UT, respectively).

### 3.2 Physiological Responses to EHS

$T_{re}$  tolerated at EXH was higher in TR ( $39.7 \pm 0.1$  °C) compared with UT ( $39.1 \pm 0.1$  °C), which produced significantly longer TT in TR ( $162.5 \pm 11$  min) compared with UT ( $106 \pm 10$  min). However, despite the longer TT for TR, the rate of increase in  $T_{re}$  ( $1.2 \pm 0.05$  °C·h<sup>-1</sup>) and time between  $T_{re}$  sampling intervals were not significantly different between groups up to 39.0°C. Although HR was lower throughout the EHS for TR, values at EXH were not different between groups at  $160 \pm 3$  b·min<sup>-1</sup> or  $81 \pm 1$  % of  $HR_{peak}$ . The absolute metabolic cost of walking at 4.5 km·h<sup>-1</sup> and 2% incline was not different between groups at  $15.2 \pm 0.1$  ml·kg<sup>-1</sup>·min<sup>-1</sup>, however, this value represented a greater relative exercise intensity for the UT ( $35.9 \pm 1.3$  %  $\dot{V}O_{2peak}$ ) compared with the TR ( $24.8 \pm 0.9$  %  $\dot{V}O_{2peak}$ ). Individual differences in BV were not related to differences in  $T_{re}$  tolerated ( $r = 0.35$ ,  $p > 0.05$ ).

### 3.3 Plasma Endotoxin and LPB

Plasma endotoxin and LBP levels were not significantly different between groups at baseline, however a significant group effect (UT > TR) was observed throughout the EHS as depicted in Figure 2. Significant increases in plasma endotoxin were first observed at a  $T_{re}$  of 38.5°C for UT and a greater than 2-fold increase in plasma endotoxin was observed for UT compared with TR at a  $T_{re}$  of 39.0°C.

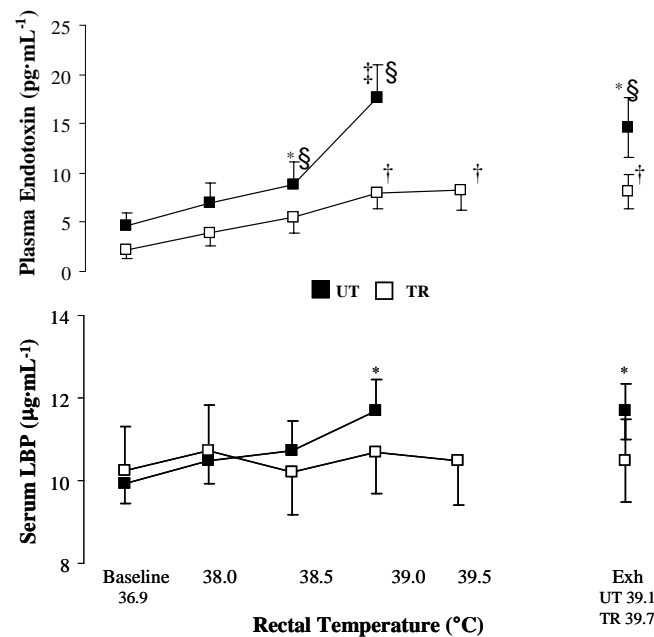


Figure 2: Circulating plasma endotoxin and serum LPS binding protein (LBP) concentrations in trained (TR) and untrained (UT) during exertional heat stress. Values are mean  $\pm$  SE. \* UT  $p < 0.05$  from baseline (B), ‡ UT  $p < 0.05$  from 38.5°C, † TR  $p < 0.05$  from baseline, § between-group significance.

### 3.4 Intracellular Cytokine Production

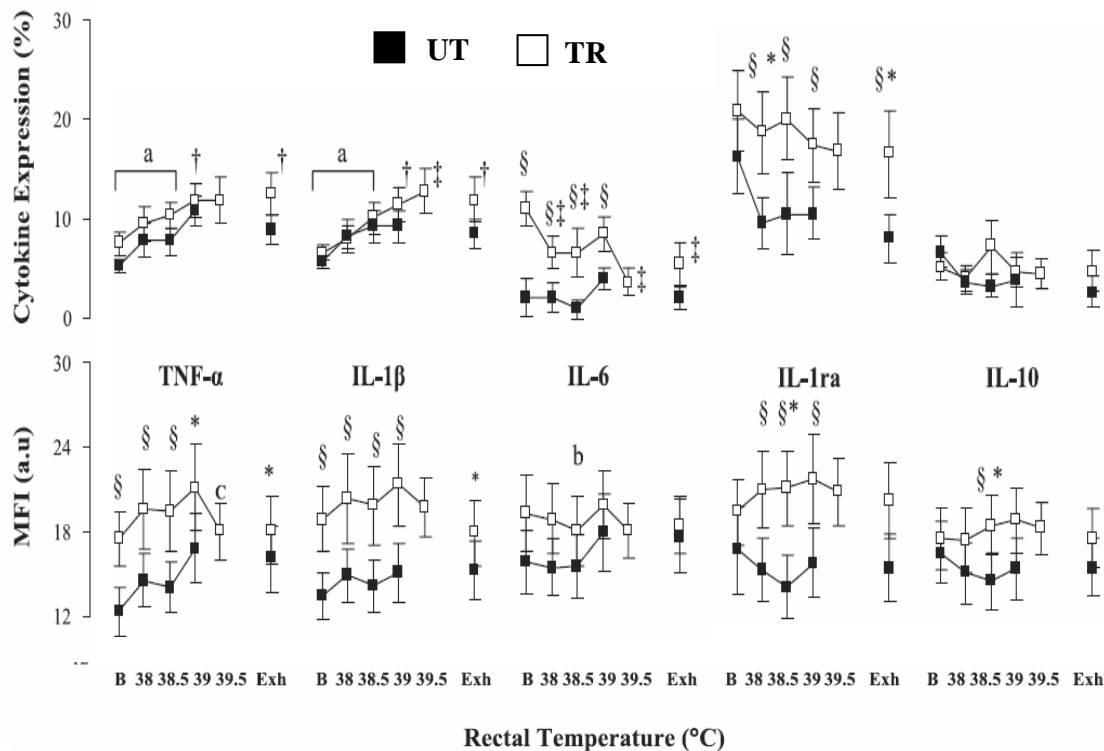
Intracellular changes in the percentage of cytokine-positive and protein content (MFI) for inflammatory monocyte subsets are shown in Figure 3. EHS produced a temperature-dependant increase in TNF- $\alpha$  and IL-1 $\beta$  expression and protein content. Of note, was the significant within-group difference in cytokine-positive cells at 39.0°C, yet MFI increases in TNF- $\alpha$  (at 39.0°C) and IL-1 $\beta$  (at EXH) were observed only in UT. In contrast, MFI was greater for TR for TNF- $\alpha$  and IL-1 $\beta$  compared with UT and TNF- $\alpha$  actually decreased from 39.0 to 39.5°C for TR. IL-6 and IL-1ra expression were elevated in TR with a temperature-dependent reductions observed during EHS in TR and UT, respectively. MFI for IL-10 decreased during EHS for UT.

### 3.5 Intracellular HSP 72

EHS produced a temperature-dependant increase in MFI for intracellular HSP72 in circulating inflammatory monocytes in TR subjects only (Figure 4). Intracellular HSP72 protein content was also significantly greater for TR compared with UT both with EHS and with *in vitro* heat shock (data not shown).

### 3.6 Cellular Apoptosis

A significant increase in the percentage of spontaneous apoptotic cells was observed only in UT subjects with EHS, whereas *in vitro* heat shock significantly increased the percentage of apoptotic cells in both groups at baseline but only in TR with the blood sample obtained at EXH (Figure 5).



**Figure 3: Intracellular changes in the percentage (%) of cytokine-positive cells and mean fluorescence intensity (MFI; in arbitrary units, a.u.) in TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-1ra, and IL-10 by inflammatory monocytes during exertional heat stress between trained (TR) and untrained (UT) groups. Values are mean  $\pm$  SE. \* UT  $p < 0.05$  from baseline (B). † TR and UT  $p < 0.05$  from baseline. ‡ TR  $p < 0.05$  from baseline. § between-group significance. a, main effect of temperature. b, main effect of group.**

## 4.0 DISCUSSION

This study presents an overview of some recent novel experiments by Selkirk et al. [19, 20] that demonstrated for the first time that endotoxin leakage can occur at core temperatures well below those typically associated with pathological conditions of a systemic inflammatory response and heat stroke [2]. Our findings suggest, therefore, that a continuum of inflammatory activation exists as body core temperature increases above 38.5°C during exertional heat stress as has been suggested by others [10, 21]. Our findings have further demonstrated that sedentary individuals are at risk of a breakdown of gut barrier integrity at lower core temperatures due to the required redistribution of blood away from the splanchnic area in their attempt to transport body heat from the core to the skin surface [16]. In addition to an increase in core temperature [2], adequate blood supply is critical for the maintenance of the gastrointestinal barrier [7, 17]. It is interesting to note, however, that heart rates for both UT and TR approximated 75-80% HR<sub>peak</sub> at the core temperature (38.5°C and 39.0°C for UT and TR, respectively) where plasma endotoxin concentrations were first noted to increase (see Figure 1). Clearly, the almost 25% greater blood volumes for our TR subjects were an important factor for maintaining gut

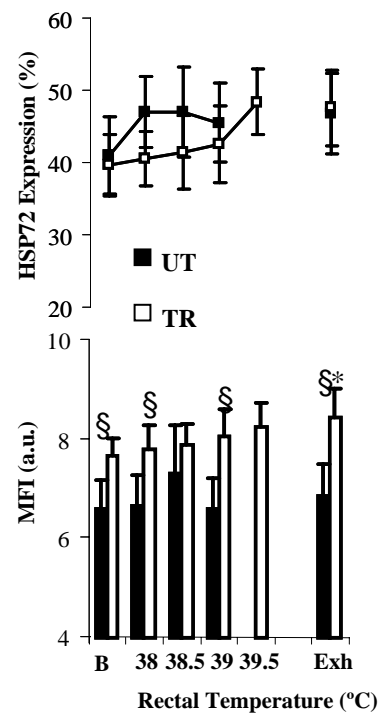


Figure 4: Changes in HSP72 intracellular expression and MFI by circulating inflammatory monocytes during exertional heat stress between trained (TR) and untrained (UT) groups. Values are mean  $\pm$  SE. \*, TR  $p < 0.05$  from baseline (B). § between-group significance.

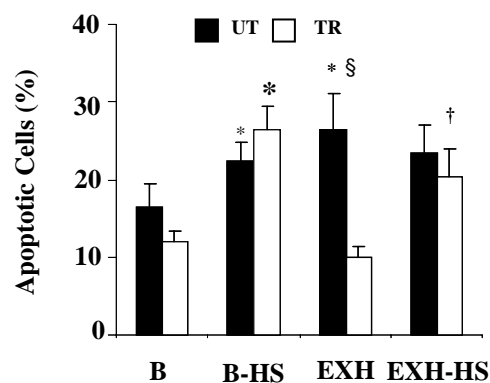


Figure 5: Changes in % of apoptotic cells in inflammatory monocytes at baseline (B) and at EXH after EHS and following *in vitro* heat shock (HS) between trained (TR) and untrained (UT). Values are mean  $\pm$  SE. \*,  $p < 0.05$  from baseline (B). †,  $p < 0.05$  from EXH. §, between-group significance.

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barrier integrity to higher core temperatures. However, this difference between groups was, by itself, unable to account for a significant proportion of the variability in thermotolerance among subjects.

There is increasing evidence that translocation of small amounts of endotoxin into the circulation occurs in healthy individuals during conditions such as heat stress, which can stimulate host defences [11]. This suggests that individuals participating in regular aerobic training may develop an improved endotoxin tolerance [3, 22] due to small, repeated exposure to LPS that results in a type of self-immunization [1]. It is also possible that this transient endotoxin translocation and the subsequent induction of the stress response may be the key cellular mediator of antioxidant and anti-inflammatory cytoprotective changes that accompany regular aerobic training. Certainly the enhanced intracellular production of anti-inflammatory cytokines IL-1ra and IL-6 and the decrease in TNF- $\alpha$  at higher core temperatures (Figure 2), the elevated intracellular HSP72 response (Figure 3) and the reduced percentage of apoptotic cells at exhaustion (Figure 4) provide direct cellular evidence of these cytoprotective changes for our endurance trained subjects. In addition, the observed impairment of HSP72 induction in the UT coincided with increased heat-stress-induced cellular apoptosis and lower  $T_{re}$  tolerated, providing an additional cross-sectional link for the benefits of endurance training, the maintenance of gut barrier integrity, and thermotolerance.

### 4.1 Operational Significance

Allied Forces are currently involved in theatres of operation that require the donning of protective clothing such as body armour as well as exposure to ambient temperatures that can reach or exceed 50°C. These conditions together with the requirements to maintain moderate to heavy work efforts for prolonged periods of time can create uncompensable heat stress where body temperature and heat storage continue to rise to dangerously high levels. The data presented here clearly reveal the advantage and importance of maintaining a high level of aerobic fitness for sustaining one's operational effectiveness for longer periods of time as core temperature increases. Thus the aerobically fit soldier will be able to march further, fight longer, and make better decisions than the unfit soldier in these extreme environments. However, our findings raise as many questions as they appear to answer. For example, will the endurance trained soldier be at risk for exertional heat injury sooner if fluid replacement can not match their elevated rates of fluid loss? Some of our additional data analyses, which are not presented here, indicate that this may be the case. Is there a threshold level of aerobic fitness associated with enhanced thermotolerance or is some regular physical activity, regardless of intensity, beneficial? Our theory regarding the importance of self-immunization with a small amount of endotoxin leakage with regular exercise implies that a certain redistribution of blood flow away from the gut is required before this leakage occurs. Our data suggest that heart rates around 75-80% of peak values must be attained before endotoxin leakage is present. In reality, it is likely that leakage occurs at somewhat lower heart rates but nevertheless these findings imply that low to moderate levels of exercise in a normothermic environment would not be sufficient to promote this self-immunization. Could the benefits of this self-immunization occur with repeated exposure to passive heating, albeit probably to higher core temperatures, and could this serve as an alternate strategy for adaptation? Perhaps the best adaptive strategy would be repeated exercise exposure in a hot environment, a process commonly referred to as heat acclimation. Those that are less aerobically fit would probably demonstrate the greatest benefits and cellular adaptations with this approach since the endurance trained individual is already considered partially heat acclimated [15]. We are currently examining these possibilities.

## **5.0 CONCLUSION**

In summary, the present findings have provided evidence to substantiate the importance of gut barrier integrity for explaining differences in thermotolerance between TR and UT. The data show the functional link between the cardiovascular adaptations that occur with endurance training and the resultant anti-inflammatory cytoprotective changes. For the soldier, maintaining a high level of aerobic fitness enables them to maintain their operational state of readiness for longer periods of time when exposed to high environmental temperatures while wearing protective clothing and carrying heavy loads.

## **6.0 REFERENCES**

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